BRIEFER ARTICLES

ARTIFICIAL PRODUCTION OF ALEURONE GRAINS

(WITH ONE FIGURE)

As is well known, aleurone grains consist mainly of protein material which may be wholly amorphous or partly amorphous and partly crystalline. In the latter case each grain consists typically of a crystal of protein (crystalloid), and an envelope of amorphous protein material whose outer layer may be differentiated from the rest. There is usually included in the envelope a globule of mineral matter or organic material combined with mineral matter (globoid). The variations occurring in different plants have been fully described by PFEFFER.[1]

Each grain is laid down in a vacuole in the protoplasm through the activity of the protoplasm itself. Its manufacture is therefore a distinctly vital process. It is the object of this paper to show that bodies of the same structure may be produced artificially. The resemblance is so striking as to leave little doubt that the essential features of the natural process have been successfully imitated.

The first step in the procedure is the preparation of protein according to the following method of OSBORNE.[2] Half a pound of *Bertholletia* nuts, after the shells have been removed, are ground into a pulp. The fatty material is then removed by repeated thorough treatments with ether, the small portion of the solvent which remains in the solid after the final decantation being allowed to evaporate completely. To the dry residue is added four or five times its volume of 10 per cent NaCl solution in which it stands some hours. Frequent shaking accelerates the dissolving of the protein. The solution of protein is then decanted and thoroughly filtered. At first the finer particles come through, but on repeated filtering through the same paper there results an absolutely clear liquid which microscopic examination shows to be without particles of any kind. This clear filtrate is placed in a dialyzer, and after some hours the sodium chloride is sufficiently removed to cause the precipitation of the protein.

Most of the protein is precipitated as clear, well formed crystals of the hexagonal system. Their thickness is usually about one-sixth

[1] PFEFFER, W., Jahrb. Wiss. Bot. **8**: 429. 1872.

[2] OSBORNE, T. B., Amer. Chem. Jour. **14**: 622. 1892.

of their width. Truncated crystals are common, especially those in which one side is about half as long as the opposite side.

Among the naked crystals are others which are furnished with an envelope (fig. 1, *a*, *b*, *g*); the whole then resembles an aleurone grain. The inclosed crystals may resemble any of the free forms, but are usually complete hexagonal crystals. They may also be of various sizes, but are usually about the size of the natural aleurone grain. Rarely more than one enters into the composition of a single grain (fig. 1, *h*), as happens also in some kinds of natural grains.

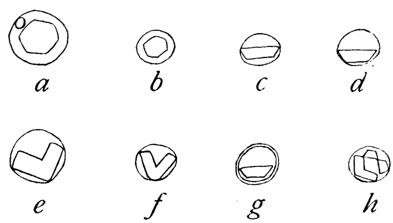

FIG. 1.—Artificial aleurone grains: *a*, protein crystal surrounded by an amorphous protein envelope in which is included a drop of oil which in size and position resembles a globoid; *b*, similar body without an oil drop; *c–h*, various forms of crystals resembling those which occur in natural aleurone grains; *g*, the amorphous envelope with a differentiated outer layer such as occurs in some natural grains; *h*, grain containing three crystals, a condition sometimes found in natural grains.

The envelope varies in thickness independently of the size of the crystal. It is usually arranged symmetrically about the latter, but the truncated crystals have a tendency to occur at one side. The outline of the grain is then globular or slightly elliptical, but not angular. Occasionally the outermost layer of the envelope differs from the rest in being more opaque and slightly granular (fig. 1, *g*), when it takes the form of a narrow but distinct membrane. This resembles the similar structure sometimes found in natural aleurone grains.

In an experiment in which some fatty matter had not been removed by the ether, many extremely small oil droplets came through the filter and were deposited with the artificial grains. A small number of these had been incorporated into the grains, each of which then consisted of a crystal, an oil droplet, and an envelope. The oil droplet thus resembled the globoid of the natural aleurone grain and the whole artificial grain was extremely similar in appearance to the natural one. In view of this it seems very probable that artificial globoids could easily be produced as inclusions in the artificial aleurone grains by causing dissolved globoid material to precipitate during the formation of the protein crystals. But this did not seem to be sufficiently important to warrant any special effort directed to this end, particularly as globoids do not always accompany the crystals in natural aleurone grains.

The yield of artificial grains varies exceedingly in different experiments. Though two experiments may be performed apparently in exactly the same manner, the number of grains obtained may be vastly different; indeed in some experiments scarcely any are produced. As a rule the first solution extracted from any given preparation gives the best results.

Chemical tests show that the grains are composed of protein, for they respond strongly to all the protein tests such as the xanthoproteic, Millon's, etc. In each test the envelope responded just as strongly as the crystal; it consists, therefore, of uncrystallized protein. With other chemical reagents their behavior is that which is to be expected; they are insoluble in water, alcohol, and sodium carbonate, soluble in weak acids and alkalies and in salt solutions. No marked difference could be observed between the solubility of the envelope and that of the crystal.

Under the action of putrefying bacteria, however, the behavior of the envelope and crystal is occasionally different. In some cases the crystal was dissolved out, leaving the ruptured envelope free; the latter then became flattened out or turned back at the edges.

In the presence of a disinfectant to prevent putrefaction, the grains usually remain unchanged indefinitely. Sometimes, however, the envelope becomes more or less angular, the angles corresponding to those of the crystal.

The proteids of other seeds were used in these experiments, but in no case could artificial grains be obtained. Castor bean, hemp, and lupine gave only crystals without envelopes.

In the case of *Bertholletia*, however, it seems evident that structures resembling the aleurone grains formed through the activity of the protoplasm have been produced in the laboratory. This imitation consists not only in reproducing what is probably the same chemical compound, but also in reproducing the same morphological structure.

In conclusion I wish to acknowledge my indebtedness to Professor W. J. V. OSTERHOUT, in whose course in plant physiology the original observation was made, and with whose advice the subsequent work was done.—W. P. THOMPSON, *Harvard University.*